بسم الله الرحمن الرحيم

Biochemical , Effects of hypolip in induced Hyperchlostrolemic Rats.

By
Islam Adam Ahamed Daffalla
B.Sc. of Veterinary Medicine (2006)
University of Nyala

Supervisor
Dr. Afaf Izzeldin Abuelgasim

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Department of Biochemistry
Faculty of Veterinary Medicine
University of Khartoum

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DEDICATION

To

My beloved parents whose love and continuous support remained for me as unlimited source of encouragement.

My husband for his great assistance and understanding during this investigation.

My brothers and sisters.

My friends.

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I would like to express my special praise and thank to Allah, who gave me the necessary health, strength and patience to conduct this work.

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ABSTRACT

The present study was carried out to investigate the biochemical effects of Hypolip, anew simvastatin drug, on serum total cholesterol,low density lipoprotein (LDL-C) ,and to assess its effects on liver function and muscle haematological and histopathological finding.

Twenty five Wister albino rats were divided into five groups. group (A) was fed basal diet only (a control group) and the other four groups (B, C, D and E) were fed 1% cholesterol-containing diet for 2 weeks to induce hypercholesterolemia. After two weeks, groups (C, D and E) were orally administrated simvastatin at a dose rate of 10, 40, and 80 mg/kg Bwt, respectively for 4 weeks. Blood samples were taken avery two weeks to investigate serum total cholesterol, LDL-C and haematological parameters. liver function test and creatine kinase were also examined.

Biopsy from liver and muscle were immediately taken after rats were slaughtered, and fixed in 10% neutral formalin, for histo-pathological investigations.

There was a significant ($P<0.05$) increase in the serum Total-cholesterol and (LDL-C) concentrations in groups B, C, D and E compared to the control A after two weeks.

The body weights significantly ($P<0.05$) increased in the induced hypercholesterolemic rat after 4 weeks.

Two weeks after administration of hypolip, there was a significant ($P<0.05$) reduction in LDL-C in groups C, D and E compared to the control B. There was a significant reduction on serum Total-cholesterol

concentration in group C only compared with control group B.

After four weeks, there was a significant decrease in serum Total-cholesterol in groups C, D and E compared with control (B). There was,also, a significant reduction in LDL-c in groups D and E compared to the control (B).

Alanine amino transfers , aspartate amino transferase and creatin kinase significantly increased in group E compared to the control B after 4 weeks. However , there were no significant changes on levels of total protein, albumin and globulin in all treated groups through out the experimental period.

After 4 weeks of administration of hypolip haematological findings revealed no significant differences in red Blood Cell counts between treated groups compared with the control group B. However, there was a significant reduction in white blood cell counts in group D haemoglobin concentrations and PCV in group E compared to control group B. There were no significant changes on platelets concentrations in all treated groups.

Histopathologically, in group B liver showed disorganization of hepatocytes and haemorrhagic. Muscle fibers were fragmented, hyalinized and necrotic. In groups C and D, the hepatocytes were vaculated and sinusoids were dilated. The muscles were hyalized and fragmented but to a lesser degree compared with those of group B. In group E, the liver showed dilatation of sinusoid and congestion of the central vein.

خلاصة الأطروحة

أجريت هذه الدارسة لتقصي الأثر البيوكيميائي لدواء الهيبولاب حديث من السمفاستاتين علي قياسات الكلسترول والبروتينات الدهنية منخفضة الكثافة وظائف الكبد والعضلات وظائف الدم والتغيرات النسيجية.

استعملت (25 فأراً) من نوع البينو وقسمت لخمس مجموعات، المجموعة (أ) أعطيت الوجبة الأساسية للفئران فقط وسميت مجموعة تحكم، المجموعات (ب، ج، د، هـ) أعطيت الوجبة الأساسية مضافاً لها ١% من مسحوق الكلسترول لكل كجم من الطعام لمدة أسبوعين وذلك لإحداث زيادة في تركيز الكلسترول في الدم.

بعد مضي أسبوعين، عولجت المجموعات (جـ، د، هـ) بعقار الهيبولاب بواسطة الفم بتراكيز١٠، ٤٠ و ٨٠ ملجم من وزن الجسم علي التوالي لمدة أربعة أسابيع.

أخذت عينات الدم كل أسبوعين لقياس مستوي الكلسترول والبروتينات الدهنية المنخفضة وقياسات وظائف الكبد وانزيم الكرياتين كاينز وظائف الدم، وأخذت أيضاً عينات من الكبد والعضلات لفحص الأنسجة المريضة بعد الذبح مباشرة ووضعها فى فورملين تركيز 1%.

أظهرت نتائج تحاليل الدم بعد أسبوعين زيادة ملحوظة في مستوي كلسترول الدم والبروتينات الدهنية منخفضة الكثافة في مجموعات (ب، جـ، د، هـ ـ) مقارنة بمجموعة التحكم (أ).

هناك زيادة ملحوظة في وزن الفئران للمجموعات (ب، جـ ، د، هـ) مقارنة مقارنة بمجموعة التحكم (أ).

بعد إعطاء عقار الهيبولاب لمدة اسبوعين أوضحت النتائج وجود نقصانا ملحوظا في تركيز البروتينات الدهنية منخفضة الكثافة في الدم خصوصا مجموعات (جـ، د، هـ) مقارنة

بمجموعة التحكم (ب)، وبينما وجدت تغيرات في مستوي الكلسترول فى المجموعة (جـ) مقارنة بمجموعة التحكم .

وبنهاية الأسبوع الرابع لاعطاء الهيبواب حدث انخفاضاً ملحوظاً للكلسترول في الـدم لمجموعات (جـ، د، هـ) مقارنة بالمجموعة (ب)، والبروتينات الدهنيـة منخفضـة الكثافـة مجموعتي (د، هـ).

سجلت نسبة الانين امينو ترانسفيريز واسبارتيت امينو ترانسفيريز والكرياتين كاينيز ارتفاعاً ملحوظاً بنهاية الأسبوع الرابع من اعطاء عقار الهيبواب خاصة مجموعـات (هـ ـ) مقارنة مع مجموعة (ب). لم تشكل نسبة البروتين الكلى والبيومين والقلبيولين تغيراً ملحوظاً بين مجموعات المعالجة (جـ، د، هـ) والمجموعة (ب) خلال فترة التجربة.

اما نتائج تحليل وظائف الدم فقد اوضحت عدم وجود تغير ملحوظ فى كريـات الـدم الحمراءبين المجموعات المعالجة ومجموعة التحكم .

سجل مجموع كريات الدم البيضاء تغيرا ملحوظا في المجموعـة(د) التـي أعطيـت العقار بجرعة 40 ملجم مقارنة بمجموعة التحكم (ب) خلال فترة التجربة.

سجل تركيز الهيموقلوبين والنسبة المئوية لحجم الـدم انخفاضـاً ملحوظـاً فـي المجموعة (هـ) مقارنة بمجموعة التحكم (ب) بنهاية التجربة .

لم تشكل الصفائح الدموية تغيرا ملحوظا فى المجموعات المعالجة ومجموعة التحكم.

عند فحص الأنسجة لحوظ عدم وجود انتظام الخلايا الكبدية نزف دمـوى,ووجـود انفصال وتكسر فى الالياف العضلية وتنكس زجـاجى ونخـر فـى المجموعـة (ب).وفـى المجموعتين (ج,د) لوحظ توسع الجيبانيات الكبدية وفراغات, وفى العضلات تنكس زجـاجى وتكسر الالياف العضلية ولكن اقل درجة مقارنة بمجموعة التحكم (ب).
وفى المجموعة (هـ) وجود توسع الجيبانيات الكبدية واحتقان الوريد المركزى .

INTRODUCTION

Lipid comprises a diverse range of molecules and to some extent is a relatively water-insoluble or no polar compounds of biological origin, including waxes, fatty acids, fatty-acid derived phospholipids, sphingolipids, glycolipids and terpenoids (retinoids and steroids). Some lipids are linear aliphatic molecules, while others have ring structures. Some are aromatic, while others are not.

Cholesterol is a lipidic, waxy steroid found in the cell membranes and transported in the blood plasma by lipoproteins of all animals (Emma, 2009).

Lipoproteins are biochemical assemblies that contain both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins (Garrett and Grisham 1995).

Disorders of lipid metabolism are manifested by elevation of the plasma concentration of the various lipid and lipoprotein fraction (Brown, 2001).

Hypercholesterolemia is, higher concentrations of Low Density Lipoprotein and lower concentrations of functional High Density Lipoproteins are strongly associated with cardiovascular disease because these promote atheroma development in arteries (Brunzell, 2008).

Simvastatin belongs to stains, is a cholesterol-lowering drug. It reduces cholesterol by inhibiting an enzyme (Hydroxy Methyl Glutaryl - CoA reductase (HMG-COA) in the liver, which is necessary for the production of cholesterol. In the blood, statins lower

total and LDL (bad cholesterol) as well as triglycerides, but increase HDL (good cholesterol). Myopathy can be a serious adverse effect even an elevated creatine kinase (CK) (Chan *et al.*, 2005).

Simvastatin may include Serum aminotransferase elevations (Tuteja *et al.,* 2008).

The objective of this study is to investigate the ability of hypolip anew manufactured drug to lower plasma total-cholesterol and LDL- cholesterol to assess the drug for its effect on enzymes activities and on the liver and muscle.

CHAPTER ONE
LITERATURE REVIEW

1.1 Lipids

Lipids are broadly defined as any fat-soluble (Lipophilic), naturally occurring substance such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids and others (Michelle *et al.*, 1993).

Lipids in human plasma are transported in form of macro-molecular complex term lipoproteins. A number of metabolic disorders that involve elevation in levels of any lipoprotein species are thus termed hyper-lipoproteinemias or hyperlipidemias. The term of hyperlipidemia don't mean increased levels of triglycerides in plasma (Malloy and Kane ,2001).

1.2 Lipoproteins

They are a biochemical assembly that contains both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins (Garrett and Grisham 1995).

1.3 Cholesterol

Is a lipidic, waxy steroid found in the cell membranes and transported in the blood plasma of all animals (Emma, 2009). It is an essential component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important precursor molecule for the biosynthesis of bile acids, steroid hormones, and is also important for

the metabolism of fat-soluble vitamins. Cholesterol is the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes, such as plants and fungi. It is almost completely absent among prokaryotes, which include bacteria (Pearson, *et al.* 2003).

1.3.1 Types of cholesterol

1.3.2 Chylomicrons

Chylomicrons are formed in the intestine and carry triglycerides of dietary origin, unspecified cholesterol, and cholesteryl esters. They transit the thoracic duct to the Blood stream (Malloy and Kane 2001).

1.3.3 Very low density lipoproteins (VLDL)

They are secreted by the liver and exported triglycerides (TG) to peripheral tissues. The hydrolysis of TG by Lipoprotein Lipase (LPL) yield free fatty acids for storage in adipose tissue and oxidation in tissues such as cardiac and skeletal muscle. Depletion of triglycerides produces remnants, intermediate low density Lipoprotein IDL, some of which are endocytosed directly by liver and the remainders are converted to low density lipoprotein by further removal of TG mediated by hepatic lipase (Martin, 2006).

1.3.4 Low density lipoproteins (LDL)

LDL is a type of lipoprotein that transports cholesterol and triglycerides from the liver to the tissues. An increased level of LDL can also result from increased secretion of its precursor VLDL as well as from decreased LDL catabolism (Malloy and Kane 2001).

1.3.5 High density lipoproteins (HDL)

They transported cholesterol from peripheral tissue to liver. Apolipoprteins of HDL, Apo (A) are secreted by the liver and intestine. HDL acquires cholesterol from peripheral tissue in a pathway that protects the cholesterol homeostasis of cells. In this pathway cholesterol is transported from the cell membrane by a transporter protein acquired by a small particle termed prebeta -1 HDL, and then esterifies by Lecithin Cholesterol Acyl transferase (LCAT) enzyme, leading to the formation of larger HDL, LDL, and chlomicron remnants with the aid of cholesterol ester (Bertram and Katzung 2004).

1.3.6 Lipoprotein (a)

This is similar in lipid composition to LDL but has higher protein content. It is thought to be an independent cardiovascular risk factor; LP (a) is a genetic variation of plasma LDL. A high level of LP (a) is an important risk factor for developing atherosclerosis prematurely. The lesions in artery walls contain substances that may interact with LP (a), leading to the buildup of fatty deposits. (Martin, 2006).

1.4 Hypercholesterolemia

Hypercholesterolemia there is, high concentrations of LDL and low concentrations of functional HDL which are associated with cardiovascular disease because they promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease (Brunzell *et al.,* 2008) LDL particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On

the other hand, high concentrations of functional HDL, which can remove cholesterol from cells and atheroma, offer protection and are sometimes referred to as "good cholesterol". These balances are mostly genetically determined but can be changed by body build, medications, food choices, and other factors (Durrington, 2003).

1.4.1 Atherosclerosis

Is the condition in which an artery wall thickened as the result of a build up of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels. It is a chronic inflammatory response in the walls of arteries, due to the accumulation of macrophage and white blood cells which has promoted by low density (especially small particle) lipoproteins. Without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries (Maton *et al.*, 1993).

1.4.2 Coronary heart disease

This refers to the failure of coronary circulation to supply adequate circulation to cardiac muscle and surrounding tissue. It is the most common form of disease affecting the heart and an important cause of premature death (Boon *et al.*, 2006)**.**

1.5 Lipid disorders

Disorders of lipid metabolism are manifested by elevation of the plasma concentration of the various lipid and lipoprotein fraction (total, LDL-C, VLDL, triglycerides, chylomicrons) and they result, predominantly, in cardiovascular disease. Classification of Hyper-lipidemias includes two types of hyperlipidemia (Brown, 2001):

1. Primary hyperlipoproteinamia: is an inherited disorder and have 5 types:
    a. Familial hypertriglyceridemia which is associated with lipoprotein lipase deficiency (LPL) .The low activity of LPL results in decreased removal of triglycerides (TG) from blood stream.
    b. Familial combined-hyperlipidemia is characterized by increased hepatic secretion of apolipoprtoein B-containing VLDL and its conversion to LDL.
    c. Familial dysbeta lipoproteinemia there is a defect in a poliopeprotein E (the major ligand), that allows subsequent metabolism of remnant particles derived from VLDL and chylomicrons.
    d. Familial hypo-alpha lipoproteinemia in which the serum concentration of HDL is low, lead to coronary heart disease (CHD) and peripheral vascular disease.
    e. Familial hypercholesterolemia characterized by elevation of total cholesterol and LDL-cholesterol in plasma. In polygenic hyperlipdemia there is an increased level of total and LDL cholesterol, but in lesser degree, which results from over production of VLDL in the liver due to a combination of high dietary fat, obesity and individual susceptibility. It is the most common disorder in adult life with atherosclerosis accruing early but with lesser degree.
2. Secondary hyperlipidemia, result from many factors including liver and biliary diseases, Obesity, hypothyroidism, diabetes

mellitus, diet, excess alcohol, renal disease and gluco-corticosteroids drugs (Brown, 2001).

1.6 Management of hyperlipidemia

Any medical disorder that may lead to hyperlipieamia e.g. diabetes, hypothyroidism should be treated first (Jonsson, 2001). There are many methods by which hyperlipoproteinemia can be managed:

1.6.1 Lipid- lowering plants

A number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia it's and related complications in patient (Dahanukar and Rege, 2000).

Phytosterols either as plant sterols or plant stanols are natural cholesterol-like substances derived from plants used to prevent hyperlipidemia (Piironen *et al*., 2000).

The main mechanism by which phytosterols reduce blood cholesterol is to inhibit cholesterol absorption in the small intestine. Therefore, the physical forms, carriers and solubilization of the phytosterols are important characteristics to determine the efficacy of phytosterols on cholesterol lowering (Berger *et al*., 2004).

Mukluk myrrh tree (Combphara mukluk), caused reduction in total cholesterol by (14-27%) and 22-30% of triglycerides levels, at seam time while HDL-cholesterol has increased by 20% (Satyavati, 1988).

The lipid lowering activity of *Autocephalous indicus* root extract has been studied in triton WR-1339 induced hyperlipidemia in rats. In this model, feeding with root extract (500 mg kg^{-1} b.w.)

lowered plasma lipids. Both lipid lowering and antioxidant activities in root extract of *A. indicus* could help prevention of hyperlipidemia and related diseases (Vishnu *et al*., 2008).

1.6.2 Lipid-lowering Drugs

In general, drugs act to reduce the concentration of cholesterol within hepatocytes, causing a compensatory increase in low-density - receptors (LDL- R) on their surface, and increased uptake of Cholesterol-rich LDL particles from the blood stream. Members of this group Includes fibrate, niacin, bile acid sequestrant and satin.

1.6.2.1 Fibrates

Fibrates, hypolipidemic agents are a class of amphipathic carboxylic acids. They are used for a range of metabolic disorders, mainly hypercholesterolemia. Members of fibrate include benzafibrate, ciprofibrate, clofibrate, gemfibrozil and fenofibrate (Wikipedia, 2004).

1.6.2.2 Mechanism

Fibrates are agonists of the peroxisome proliferators activity receptor alpha (PPAR-α) in muscle, liver and other tissues. Activation of PPAR-α signaling result in increased β-oxidation in the liver, decreased hepatic triglycerides secretion, increased lipoprotein lipase activity (Wikipedia, 2004).

1.6.2.3 Indications

Fibrates are used as accessory therapy for hypercholesterolemia, usually in combination with statins. Clincal trials do support its use as a monotherapy agent. Fibrates increase HDL levels and decrease triglyceride levels (Wikipedia, 2004).

1.6.2.4 Side effects

Most fibrates can cause mild stomach upset and myopathy .Since fibrates increase the cholesterol content of bile, they increase the risk for gallstones. It also causes an increased risk of rhabdomyolysis (Wikipedia.org/wiki/fibrate"2004).

1.6.3 Niacin

Niacin (B3) is a water-soluble vitamin which prevents the deficiency disease pellagra. It is an organic compound with the molecular formula C6H5NO2.Itis a derivative of pyridine, with a carboxyl group (COOH) at the 3- position. Niacin is converted to nicotinamide and then to NAD and NADP in vivo. Niacin is a precursor to NADH, NAD, NAD+, NADP and NADPH, which play essential metabolic roles in living cells (Cox *et al.* 2000).

1.6.3.1 Mechanism

Niacin acts as inhibitor of VLDL secretion, this in turn decreasing production of LDL. Increased clearance of VLDL via Lipoprotein lipase pathway contributes to triglycerides reduction, thus the catabolic rate of HDL decreased (NCEP, 1985).

1.6.3.2 Indications

Niacin, like fibrates, lowering triglycerides by 20-50%. It may also lower LDL by 5-25% and increase HDL by 15-35% (NCEP, 1985).

1.6.3.3 Side effects

Niacin may cause hyperglycemia, and may cause liver damage (NCEP, 1985).

1.6.4 Bile acid sequestrants

Bile acid sequestrants are a group of medications used for binding certain components of bile in the gastrointestinal tract. They are generally classified as hypolipidemic agents. Three synthetic polymeric resins are members of this class namely Cholestyramine, Colestipol and Colesevelam (Wong, 2001).

1.6.4.1 Mechanism

Bile acid sequestrants are polymeric compounds which serve as ion exchange resins. They exchange anions such as chloride ions for bile acids; thus bind bile acids and sequester them from enterohepatic circulation (Wong , 2001).

1.6.4.2 Indications

Bile acids are biosynthesized from cholesterol, hence the disruption of bile acid reabsorption will decrease cholesterol level, particularly low density lipoprotein. Therefore, they may be used for the treatment of hypercholesterolemia and dyslipidmia (Wong , 2001).

1.6.4.3 Side effect

Since bile acid sequestrants are designed to stay in the gut, they generally do not have systemic side effects. However, they may cause problem in the gastrointestinal tract, such as constipation, diarrhea, and flatulence (Wong, 2001).

1.6.5 Statins

Statins are the treatment of choice for management of hypercholesterolemia because of their efficacy and safety profile. They also have an increasing role in managing cardiovascular risk patients with relatively normal levels of plasma cholesterol

(Schashtrer , 2004). This group included Atrovastatin, Cerivastatin, Fluvastatin, Lovastatin, Mevastatin, Pitavastan Pravastatin, Rosuvastatin and Simvastatin.

1.6.5.1 Mechanism

Commonly lipid-modifying therapies are statins which (HMG-CoA reductase inhibitors). HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate, the rate-limiting step in de novo cholesterol synthesis. Competitive inhibition of this enzyme by the statins decreases hepatocyte cholesterol synthesis. The associated reduction in intracellular cholesterol concentration induces LDL-receptor expression on the hepatocyte cell surface, which results in increased extraction of LDL-C from the blood and hence decreased circulating LDL-C concentration (Hobbs *et al.*, 1992).can also reduce levels of atherogenic lipoproteins by inhibition of hepatic synthesis of apolipoprotein B100 and a reduction in the synthesis and secretion of triglyceride-rich lipoproteins (Grundy, 1998) (Fig. 1).

1.6.5.2 Indications

Statins, the most potent cholesterol-lowering agents available, lower LDL cholesterol. This translates in a 60% decrease in the number of cardiac events and a 17% reduced risk of stroke (Liu *et al*., 2006).

1.6.5.3 Side effects

Statins are generally well-tolerated and have only two major side effects. That occurs, relatively rarely, raised liver enzymes and skeletal muscle damage. More serious but rare reactions include myositis and myopathy, with the potential for rhabdomyolysis (Marcoff and Thompson 2007).

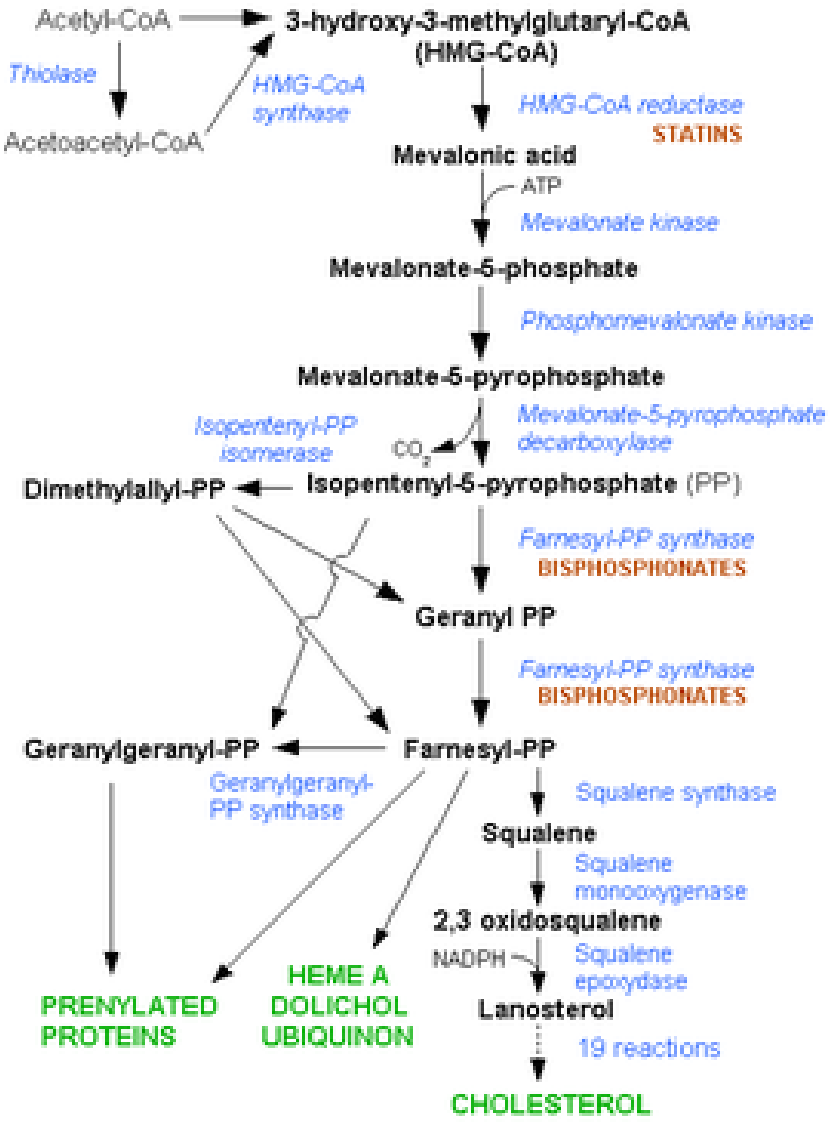


Fig. (1): The HMG-CoA reductase pathway, which is blocked by statins via inhibiting the rate limiting enzyme HMG-CoA reductase

File: HMG-CoA reductase pathway.png

From Wikipedia, the free encyclopedia

Combining any statin with a fibrate, another category of lipid-lowering drugs increases the risks for rhabdomyolysis (Graham, 2005). Consumption of grapefruit or grapefruit juice inhibits the metabolism of statins; furanocoumarins in grapefruit juice inhibit the cytochrome P450 enzyme CYP3A4, which is involved in the metabolism of most statins (however it is a major inhibitor of only lovastatin, simvastatin and to a lesser degree atorvastatin) (Kane and Lipsky, 2000).

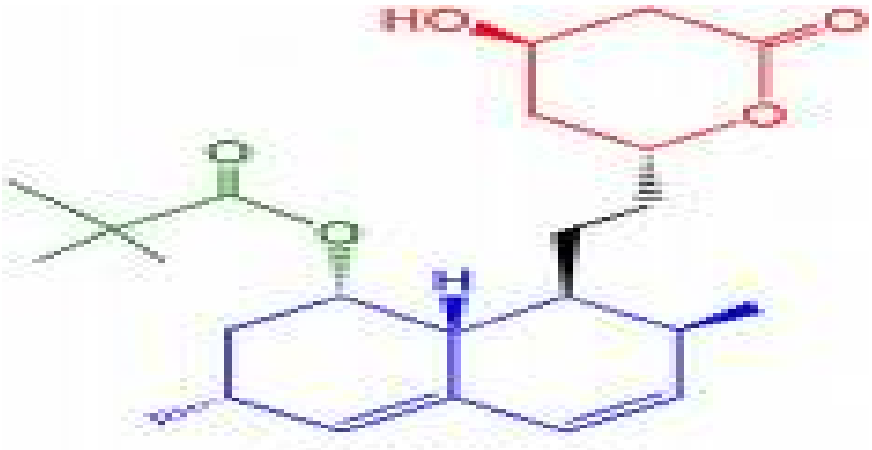
1.6.6 Hypolip (Simvastatin)

It is a hypolipidemic drug belonging to the class of "statins". It is used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease. Simvastatin is a synthetic derivate of a fermentation product of *Aspergillums terreus*.

Simvastatin is a cholesterol-lowering medication that blocks the production of cholesterol) in the body. Simvastatin reduces low-density lipoprotein (LDL) cholesterol and total cholesterol in the blood. Lowering cholesterol can help prevent heart disease and hardening of the arteries, conditions that can lead to heart attack, stroke, and vascular disease.

1.6.6.1 Description

Simvastatin is butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4hydroxy-6-oxo-2*H*-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1*S*-[1α,3α,7β,8β(2*S**,4*S**),-8aββ]]. Simvastatin is a white, no hygroscopic, crystalline powder that is practically insoluble in water and freely soluble in chloroform, methanol and ethanol. The empirical formula of simvastatin is $C_{25}H_{38}O_5$ and its molecular weight is 418.57 (Fig. 2).



Simvastatin

Figure (2): Chemical Structure of Simvastatin

1.6.6.2 Pharmacokinetics

Simvastatin is a lactone that is readily hydrolyzed in vitro to the corresponding B-hydroxyacid,a potent inhibitor of HMG-CoA reductase.Following an oral dose of tables simvastatin in man 13% of the dose was excreted in urine and 60% in feces.Both simvastatin and B- hydroxyacid metabolite are highly bound plasma protein. The major active metabolites of simvastatin present in plasma are the B-hydroxyacid of simvastatin and its 6'-hydroxy, 6'-hydroxymethyl, and 6'-exomethylene derivatives.

1.6.6.3 Indications

Simvastatin is indicated to reduce elevated total-cholesterol, LDL-cholesterol, Apo B, and TG, and to increase HDL-C in patients with primary hypercholesterolemia, treat patients with primary dysbetalipoproteinemia and reductions in risk of CHD mortality and cardiovascular events.

1.6.6.4 Side effects

Common side effects (>1% incidence) may include abdominal pain, diarrhea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps (Gen, 2007). Serum aminotransferase elevations are a commonly known adverse effect of simvastatin therapy (Tuteja, 2008). Myopathy can be a serious adverse event elevated creatine kinase (CK) (Chan *et al.*, 2005).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental Animals

Twenty five healthy Wister albino rats of both sex weighing from (100-230gm) were supplied by Medicinal Aromatic Plant Research Institute (MAPRI), National Research center. They were housed under standard conditions within the premises of (MAPRI) and have free access to water and standard diet. They were left for 7 day as and adaptation period.

2.1.2 The diet

The basal diet which provided the nutritional requirements to rats was composed of the following constituents (per Kg):

Wheat flour	660
Dry meat	147
Plant oil	120
Sodium chloride	3g

2.1.3 Hypolip (Simvastatin)

The powder form of the drug was supplemented by Blue Nile Pharmaceutical Factory. The different concentrations of the drug were prepared by dilution of the powder .The stock solution was prepared by dissolving 100mg simvastatin in 100ml distil water, The solutions were stored in refrigerator to be used at the time of administration. The concentration of simvastatin was 1mg/ml, and then the doses were calculated according to rat's weight.

2.1.4 Cholesterol

The powder form of 1% cholesterol was supplemented from Lab Line Company and was added to the rat's basal diet to induce hypercholesterolemia according to the method of Birkner *et al.*, (2009).

2.1.5 Experimental Design

The rats were divided randomly into five groups (5/rats). Group A was left as control, group B, C, D and E were fed a diet containing 1% cholesterol for 2 weeks only so as to induce hyper chlesterolemia .The groups C and D administrated simvastatin using nasogastric tube daily at concentration rate of 10, 40 and 80 mg / kg Bwt respectively for 30 days.

2.1.6 Parameters

Hematological investigation included red blood cells (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV) and hemoglobin concentration (Hb).

Serum investigation included, total serum protein concentration, albumin concentration, globulin concentration ,serum total cholesterol, low density lipoprotein, alanine amino transferase (ALT) activity aspartate amino transferase (AST) activity and creatine kinase (CK) activity. Samples were taken from liver, and muscle at the end of the experimental period and fixed in 10% natural buffered formalin for histopathological investigation.

2.2 Methods

2.2.1 Blood collection

Blood was collected by puncturing retro-orbital plexus, with heparinized capillary tube, and allowed to clot at room

temperature for 30 minutes then centrifuged (Hittich EBA 35) at 300r –pm sera were separated and stored at –20^{o}C until for biochemical investigation.

Blood constituent were determined using Sysmex ®Kx-21N. It was fully automated computerized. The KX-21N employs three detector blocks and two kinds of reagent for blood analysis. The WBC count is measured by WBC detector block using the detector cont detection method. The RBC count and platelets are taken by the RBC detector block The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method.

2.2.2 Serum analysis

Sera constituents were determined using Roche Diagnostics Hitachi 902 Analyzer. It was fully automated, computerized and performs potentiometric and photometric assay.

2.2.2.1 Measurement of serum total cholesterol (TC)

Reagents

Reagent composition:	Final concentrations
Good's Buffer PH6.7	80 mmol/L
Phenol	5mmol/L
4. Aminoantipyrine	0.3mmol/L
Cholesterol esterase (CHE)	≥ 200 V/L
Cholesterol oxidase (CHO)	≥ 50 V/L
Peroxidase (POD)	≥ 3 k/L

Principle:

Cholesterol ester + H2O $\xrightarrow{CHE}$ cholesterol + fatty acids

Cholesterol + O2 $\xrightarrow{CHO}$ cholesterol -3-one + H2O2

2H2O2+phenol +4Aminoantipyrine $\xrightarrow{POD}$ Quinoneimine (Pink color) +4H2O

The intensity of the pink/red color of the Quinoneimine is proportional to the cholesterol concentration in the sample (Roeschlau *et al.*, 1974). The test has been developed to determine cholesterol concentrations with in a measuring range from 3-800 mg/dL (on Hitachi).

Procedure:

500 µL of plasma samples were put in Hitachi sample cups. The sample cups were entered in a specific place on Hitachi 902 and then the parameters to be measured were selected from the touch screen and registered automatically. Thereafter the machine was put on and the results were printed in ten minutes. Hitachi 902 machine adds 500µL from sample and reagent, and the reaction proceeds automatically.

Calculations:

The analyzer automatically calculates the analyze concentration of each sample and the results appear directly on the LCD touch screen at the end of the reactions between the samples and reagent.

$$\text{Cholesterol concentration mg/dl} = \frac{\text{O.D of the test}}{\text{O.D of the standard}} \text{ X Standard concentration}$$

2.2.2.2 Measurement of serum low Density Lipoprotein (LDL)

Reagent Composition:

Reagent (1)	final concentration
Good's Buffer PH6.8	25 mmol/L
Cholesterol Esterase (CHE)	5000 U/L
Cholesterol Oxidase (CHO)	5000 U/L
N-(2-Hydroxy-3-sulfopropyl)-3,5dimethoxyaniline (HDAOS)	0.64 mmol/L
Castalase	1000 KU/L

Reagent (2)

Good's Buffer PH 7.0	25mmol/L
4Aminoantipyine	3-5 mmol/L
Peroxidase (POD)	20KU/L
Soium azide	

Principle:

In this method, non-LDL lipoproteins are enzymatically processed, while LDL is selectively protected in the first incubation step with reagent (I). In the second step, LDL is released and selectively determined (Bachorik, 1997).

LDL + protecting reagent (reagent 1) ⟶ protected LDL.

HDL, VLDL, chylomicrons $\xrightarrow{\text{CHE, CHO}}$ cholestenone H_2O_2

HDL.C CHE, CHO cholestenone + H_2O_2

H_2O_2 + 4.Aminoantipyrine+H-DAODS $\xrightarrow{\text{POD}}$ (blue colored complex)

The test has been developed to determine LDL concentrations within a measuring range from 1-400 mg/dl.

Procedure: It is similar to that used in cholesterol measurement

Calculation:

It is similar to that used in cholesterol measurement

2.2.2.3 Alanine amino Transferase (ALT)

This enzyme is formerly known as Glutamate Pyruvate Transaminase (GPT). The assayed of GPT as recommended by the international federation of clinical chemistry (IFCC) (Clin. *et al.*, 1980).

Principle:

NADH is oxidized to NAD; the resulting decrease in absorbance at 340 nm is directly proporational to the activity of GPT in the sample.

L – alanine + 2- oxoglutarate $\xleftrightarrow{GPT}$ pyruvate + L-Glutamate

Pyruvate + NADH + H $\xleftrightarrow{LDH}$ L- lactate + NAD

NAD = Nictinamid Adenine Dinucleotide

NADH =Reduced NAD

LDH = Lactate Dehydrogenase

GPT = Glutamate-Pyruvate Transaminase

Procedure:

Two ml of alanine amino transferase and control were pipetted into two separated test tubes and were put into control and calibration positions in to Hitachi apparatus automatic analyzer model 902. Calibration was according to the identification number (ID) for the alanine amino transferase. The reading at wave length 902 nm the GPT was measured in (U/L)

2.2.2.4 Aspartate amino transferase (AST)

Glutamic oxaloacetate (GOT) in sera was measured by international Federation Clinical Chemistry (Clin *et al.,* 1980).

Principle:

NADH is oxidized to NAD; the resulting decrease in absorbance at 340 nm is directly proportional to the activity of GOT in the sample.

L – Aspartate + 2- oxoglutarate $\xleftrightarrow{G0T}$ Oxaloacetate + L-Glutamate

Oxaloacetate +NADH +H $\xleftrightarrow{MDH}$ L-malate +NAD

GOT=Glutamate Oxaloacetate Transaminase

MDH=Malate Dehydrogenase

NAD=Nicotinamide Adenine Dinucleotid

NADH= reduced NAD

Procedure:

Two ml of aspartate amino transferase and control were pipetted into two separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer model 902 .Calibration was according to the identification number (ID) for the aspartate amino transferase. The reading at wave length 902 nm .the AST was measured (IU/L).

2.2.2.5 Creatine Kinase (CK):

The activity of (CK) was measured by an optimized DGKC/IFCC (Wurzburg *et al.*, 1977).

Principle:

Creatine phosphate + ADP $\xleftrightarrow{CK}$ creatine + ATP

ATP+ Glucose $\xleftrightarrow{HK}$ ADP+ Glucose 6-p (G-6-P)

G-6-p+NADP $\xleftrightarrow{G\text{-}6\text{-}P\text{-}DH}$ Gluconate -6-p+NADH+H

CK= Creatine kinase.

HK= Hesokinase.

G-6-P-DH=Glucose -6-phosphate dehydrogenase.

Procedure:

Two ml of creatine kinase and control were pipetted into separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer. Calibration was according to the identification number (ID) for the creatine kinase. The reading at wave length 902 nm the CK was measured in (U\L).

2.2.2.6 Total protein determination

The determination of total protein concentration was done according to Biuret method described by (Johnson *et al.*, 1999).

Principle:

The principle of this total protein assay is the biuret reaction. In alkaline solution, cupric ions react with all compounds with two amide or peptide bonds linked either directly or through an intermediate carbon atom to form a violet colored complex.

Procedure:

Two ml of Protein and control were pipetted into two separated test tubes and were put in to control and calibration positions into Hitachi apparatus automatic analyzer. Calibration was according to the identification number (ID) for the protein. The reading at wave length 909nm the total protein measured in (g\dll).

2.2.2.7 Albumin

Albumin concentration was measured using Bromo Cresole Green (BCG) method described by (Doumas *et al.*, 1975).

Principle:

The procedure is based on the binding of brom cresol green (BCG) to albumin. The intensity of the blue-green color produced in the reaction is proportion to the concentratio**n** of albumin in the sample.

Procedure:

Two ml of albumin and control were pipetted into two separated test tubes and were put in to control and calibration positions into Hitachi apparatus automatic analyzer. Calibration was done according to the identification number (ID) for the albumin. The reading at wave length 902 mn. Protein was measured in (g/dl).

2.2.2.8 Globulin

This parameter is obtained by subtracting albumin concentration from total protein concentration.

2.2.2.9 Total bilirubin

Principle

Total bilirubin reacts with diazotized dichloroaniline to form a colored Azocomoned (Tolman and Rej 1999).

Procedure:

Two ml of bilirubin and control were pipetted in to two separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer model 902 . Calibration was according to the identification number (ID) for the bilirubin. The wave length 902 nm the bilirubin was measured in (mg/dl).

2.3 Histopathological methods

At the end axepariment rats were slaughtered, specimens from Liver and Muscle. Were immediately removed and fixed in 10% neutral Formalin (sodium hydrogen 6.5 gm/L and sodium dehydrogenate 4.0 gm/L0. They were embedded in paraffin wax, and sectioned at 5µm and stained by Haemotoxyline and Eosin (H & E) using (Druray and Wallington, 1980).

2.4 Statistical analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) by Completely Randomized Design (CRD), and the data were presented as means ±standard errors (SE) according to (MSS, 2003).

CHAPTER THREE
RESULTS

3.1 Induction of hypercholesterolemia

The effects of feeding 1% cholesterol diet on plasma total cholesterol and LDL-c in albino rats for 2weeks is presented in table (1).

The plasma levels of Total cholesterol (T.C) concentrations showed a highly significant increase ($P<0.01$) in groups (B, C, D and E) fed 1% cholesterol diet compared to control (A) after two weeks of experiment.

The plasma levels of Low Density Lipoprotein- cholesterol (LDL-C) showed highly significant increase ($P<0.01$) in groups (B, C, D and E) fed 1% cholesterol diet compared to control (A) after 2 weeks.

3.2 Body weight

The mean changes on body weight of rat feed 1%cholesterol diet and the control group were shown in table (2).

There were significant differences ($P<0.05$) between control and rats feed 1% cholesterol diet throughout experimental period in the body weight. The weight of rats fed cholesterol was higher by 15.8, 20%.

3.3 Serum lipid profiles

3.3.1 Total cholesterol (T.C)

The effects of oral administration of simvastatin on T.C in an induced hypercholesterolemic rat are presented in table (3).

Table (1): Mean changes in Total cholesterol and Low Density Lipoprotein cholesterol in an induced hyper-cholesterolemic rat.

Period (weeks)	Groups	Total cholesterol (mg\dl)	LDL-c (mg\dl)
2	A	52.75 ± 3.6	12.07±1.7
	B	155.33 ± 7.2*	91.60±4.8*
	C	122.50 ± 5.5*	69.50±4.2*
	D	193.33 ±8.1*	132.97±6.7*
	E	128.00 ± 5.7*	71.58±4.2*

Values are presented as Means ± SE (standard errors)
* ($P<0.01$)
Group (A): Control (fed normal diet only).
Groups (B-E): 1%cholesterol-containig died.

Table (2): Mean changes in body weight in an induced hyper-cholesterolemic rat.

Groups	Initial Body weight (gm)	4week
A	138±5.25	164.5±6.40
B	138±5.25	172.5±6.56*
C	137±5.23	171.5±6.54*
D	138±5.25	172.5±6.56*
E	138±5.25	172.5±6.56*

Values are presented as Means ± SE (standard errors)
* ($P<0.05$)
Group (A): control (Fed normal diet only)
Groups (B-E): 1% cholesterol containing died.

Table (3): Mean changes in T.C and LDL-c in an induced hyper-cholesterolemic rat after 2 and 4 weeks treated with Simvastatin:

Period (weeks)	Groups	T.C(mg\dl)	LDL-c(mg\dl)
2	B	79.75 ±4.5	41.47±3.2
	C	68.75 ±4.1*	26.95±2.6*
	D	84.00 ± 4.6	22.10±2.4*
	E	77.00 ± 4.4	25.43±2.5*
4	B	107.7 ±5.2	46.47±3.4
	C	86.33 ± 5.4*	39.47±3.6
	D	82.00 ± 4.5*	31.00±2.9*
	E	55.67 ± 3.7**	33.80±3.4*

Values are presented as Means ± SE (standard errors)
Significant * ($P<0.05$)
Highly significant ** ($P<0.01$)
Groups:
(B): Fed cholesterol-containing diet 1% only.
(C): Fed cholesterol-containing diet 1% &received 10 mg/kg simvastatin.
(D): Fed cholesterol-containing diet 1%& received40 mg/kg simvastatin.
(E): Fed cholesterol-containing diet 1%& received 80 mg/kg simvastatin

There was a significant decrease (P<0.05) in plasma Total Cholesterol (T.C) in group(C) received 10 mg/kg Bwt of Simvastatin (68.75±4.1 mg\dl) compared to control (B) (79.75±4.5 mg/dl) after 2 weeks administration of Simvastatin. However, there were no significant changes in groups (Dand E) received 40, 80 mg/kg compared to control (B). Mean while there was a highly significant reduction (P<0.01) on plasma T.C in group (E) received 80mg/kg Bwt of Simvastatin (55.67±3.7 mg/dl) compared to control (B) (107.7 ±5.2mg/dl) after 4 weeks administration of Simvastatin. There was significant reduction in group (C and D) which received 10, 40 mg\Kg Bwt respectively compared to control (B).

3.3.2 Low Density Lipoprotein-cholesterol (LDL-c)

The mean changes on plasma LDL-c in an induced hyper-cholesterolemic rat treated with of simvastatin is presented in table (3).

There was a significant reduction(P<0.05) in plasma LDL-c in groups (C, Dand E) that received 10, 40,80mg/kg Bwt of simvastatin (26.95±2.6, 22.10±2.4, 25.43±3.2mg\dl) respectively compared to control (B) (41.47±3.2mg\dl) after 2 weeks administration of simvastatin. However after 4 weeks, there was a significant reduction (P<0.05) on LDL-c in groups (D and E) while received 40, 80 mg/kg Bwt simvastatin (31.00± 2.9, 33.80±3.4 mg/dl) compared to control (B) (46.47±3.4 mg\dl) but there were no significant differences on LDL-c in groups (C) received 10mg/kg Bwt of simvastatin.

3.4 Changes in serum constituents

The effects of simvastatin on concentration of activity of alanine transaminase, aspartate amino transferase and creatine kinase are given in table (4).

Table (4): The mean changes in serum constituents in an induced hypercholesterolemic rat after 2 and 4 weeks treated with simvastatin

Duration (week)	Groups	ALT(U/L)	AST (U/L)	CK(U/L)
2	B	16.06±4.1	48.62±13.7	21.0±4.5
	C	15.81±3.9	35.62±5.9	11.5±3.3*
	D	15.12±3.8	35.43±5.9	22.0±4.6
	E	22.14±4.7*	69.75±8.3*	
4	B	14.68±3.8	44.43±6.6	886.0±29.9
	C	15.75±3.9	45.43±6.6	811.0±28.4
	D	12.62±3.5	47.12±6.8	462.0±21.49*
	E	21.18±3.7*	49.75±6.9*	2300±47.95*

Values are presented as Means ± SE (standard errors)
* ($P<0.05$)
Groups:
(B): Fed cholesterol-containing diet 1% only.
(C): Fed cholesterol-containing diet 1% &received 10 mg/kg Simvastatin.
(D): Fed cholesterol-containing diet 1%& received 40 mg/kg Simvastatin.
(E): Fed cholesterol-containing diet 1%& received 80 mg/kg Simvastatin

There was a significant increase (P<0.05) between control (B) and group (E) with treated 80mg/kg Bwt of simvastatin on Alanine amino transferase (ALT) activity after 2 and 4 weeks.

On the other hand there were no significant changes between control and treated groups at doses of 10, 40 mg\kg Bwt of simvastatin.

There was a significant increase (P<0.05) between control (B) and group (E) treated 80mg/kg Bwt on aspartate amino transferase (AST) activity after 2 and 4 weeks.

There was a significant decrease (P<0.05) on serum (CK) activity in group (C) treated with 10 mg/kg Bwt and control group (B) after 2weeks.

However a significant reduction (P<0.05) on serum (CK) activity in group (D) treated with 40 mg/kg Bwt and control group (B) after 4 weeks.

There was a significant increase (P<0.05) between control (B) and group (E) treated 80mg/kg Bwt simvastatin on Creatin kinase.

3.5 Other Biochemical Tests

The effects of various doses of simvastatin on concentration of Total bilirubin, Total protein, Albumin and Globulin are given in table (5).

There were non significant differences on total bilirubin, total protein, albumin and globulin concentrations between treated rats (C, D and E) and control (B) through out experimental period.

Table (5): The mean changes in Liver Function Tests in an induced hypercholesterolemic rat after 2 and 4 weeks treated with simvastatin

Duration (week)	Groups	Total Bilirubin Mg/dL	Total Protein g/dL	Albumin g/dL	Globulin g/dL	Alb/Glb ratio
2	B	0.01±0.11	1.8±13	0.8±0.9	0.96±0.9	0.8±1
	C	0.02±0.15	1.9±1.3	1.01±1.0	0.92±0.9	1.0±1.1
	D	0.03±0.17	2.0±1.4	1.0±1.0	1.0±1.0	1.0±1.0
	E	0.03±0.19	2.0±1.4	0.9±0.9	1.0±1.0	0.9±0.9
4	B	0.05±0.2	1.8±1.3	1.0±1.1	0.8±0.9	1.2±1.2
	C	0.05±0.2	1.9±1.3	0.9±0.9	0.9±0.9	1.0±1.0
	D	0.05±0.2	1.7±1.3	1.0±1.0	0.7±0.8	1.4±1.2
	E	0.05±0.2	1.6±1.2	0.6±0.8	0.9±0.9	0.7±0.8

Values are presented as Means ± SE (standard errors)
Groups:
(B): Fed cholesterol-containing diet 1% only.
(C): Fed cholesterol-containing diet 1% &received 10 mg/kg Simvastatin.
(D): Fed cholesterol-containing diet 1%& received 40 mg/kg Simvastatin.
(E): Fed cholesterol-containing diet 1%& received 80 mg/kg Simvastatin

3.6 Hematological findings

3.6.1 Red Blood Cell Counts (RBCs)

The effect of simvastatin on hematological parameters is presented in table (6).

There were no significant differences in Red Blood Cell Counts between treated groups (C, D, and E) compared to control group (B) after 4 weeks of administration of Simvastatin.

3.6.2 White Blood Cell Counts (WBCs)

There was a significant decrease ($P<0.05$) in WBCs in group (D) received 40mg/kg Simvastatin ($8.100\pm1.4 \times 10mm^3$) compared to control (B) ($16.733\pm2.4)10mm^3$) after 4 weeks of administration of Simvastatin in hypercholesterolemic rats. However the WBCs showed no significant changes in groups (C and E) received (10, 40mg/kg) Bwt respectively compared to control (B).

3.6.3 Hemoglobin concentrations (Hb)

There was a significant decrease ($P<0.05$) in (Hb) concentration in group (E) received 80mg/kg Bwt ($7.850\pm1.4 \times 10g/dL$) compared to control (B) (11.450 ± 1.7 g/dL), however, there were no significant differences in hemoglobin concentration in groups (C and D) compared to control (B).

3.6.4 Packed Cell Volume (PCV %)

There was a significant decrease ($P<0.05$) on PCV value in group (E) by (27.10 ± 2.6) compared to control (B) (38.48 ± 3.1) after 4 weeks. However, there were no significant changes in groups (C and D) compared to control (B) after 4 weeks of experiment.

Table (6): Mean changes in hematological parameters in an induced hypercholeserolemic after 4 weeks rats treated with Simvastatin

Duration	Group	RBCs $\times 10mm^6$	WBCs× $10mm^3$	Hb g/dL	PCV %	Platelets mg/dL
4	B	6.7525±1.3	15.700±2.0	11.450±1.7	38.48±3.1	878.75±14.8
	C	5.8066±1.4	16.733±2.4	11.033±1.9	36.53±3.5	935.33±17.7
	D	4.8675±1.1	8.100±1.4*	9.425±1.5	30.43±2.8	694.25±13.2
	E	4.1175±1.0	14.850±1.9	7.850±1.4*	27.10±2.6*	870.75±14.8

Values are presented as Means ± SE (standard errors)
Significance * ($P<0.05$)
Groups:
(B): fed cholesterol-containing diet 1% only.
(C): fed cholesterol-containing diet 1% &received 10 mg/kg simvastatin.
(D): fed cholesterol-containing diet 1%& received 40 mg/kg simvastatin.
(E): fed cholesterol-containing diet 1%& received 80 mg/kg simvastatin.
RBCs: Red Blood Cell Counts **WBCs**: White Blood Cell Counts
Hb: Hemoglobin concentrations **PCV**%: Packed Cell Volume%

3.6.5 Platelets

There were no significant changes in groups (C, D and E) compared to control (B) after 4 weeks of experiment.

3.7 Histopathological Finding

In group **(**B**)** which was fed cholesterol 1% the liver showed haemorrhage, disorganization and vaculation of sinusoid fig (3). The muscle showed fragmentation , necrosis , hyalinosis, muscle banding separated each other and muscle fiber loss there nucleus (4).

In the group treated with 10mg\kg Bwt the liver was showed vaculation, muscle banding separated.

In the group treated with 40mg\kg Bwt the liver was showed dilatation of sinusoid. Muscle showed fragmentation of muscle (5).

In the group treated with 08mg\kg Bwt the liver was dilatation of sinusoid and congestion of the central vein (6)

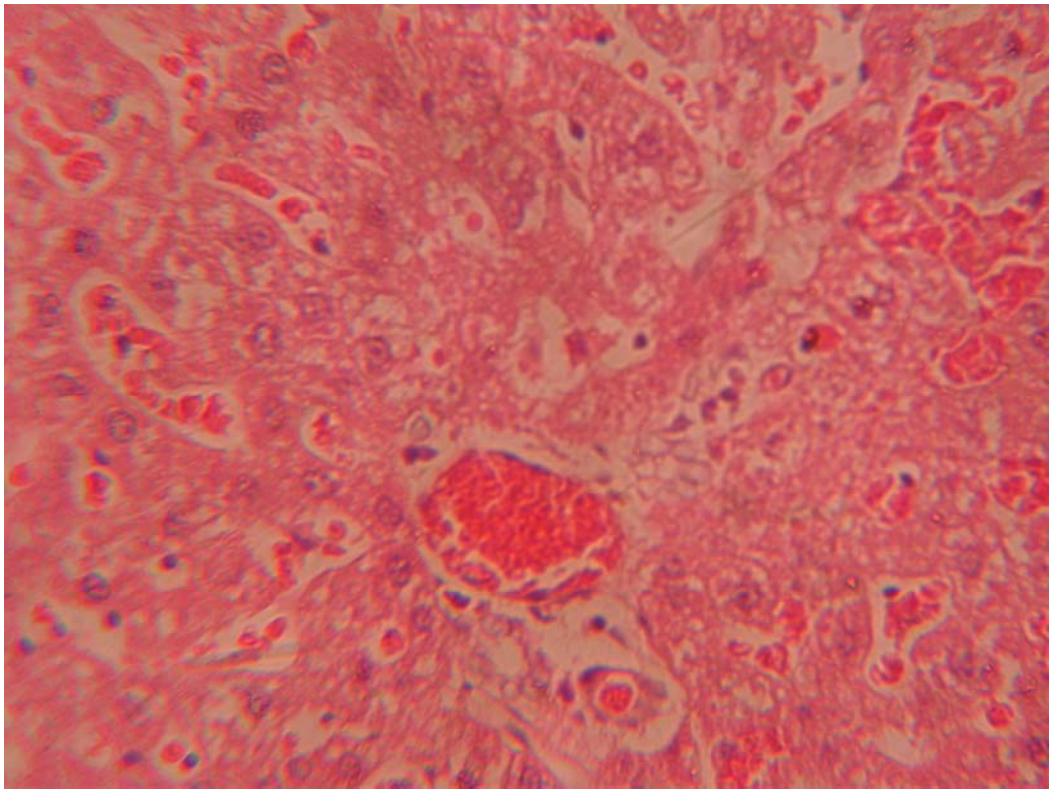


Fig (3): Liver from rats fed (1% cholesterol diet).Notice, hemorrhage, disorganization of hepatic plates and vaculization. Haemotoxaline & Eusin(400 X)

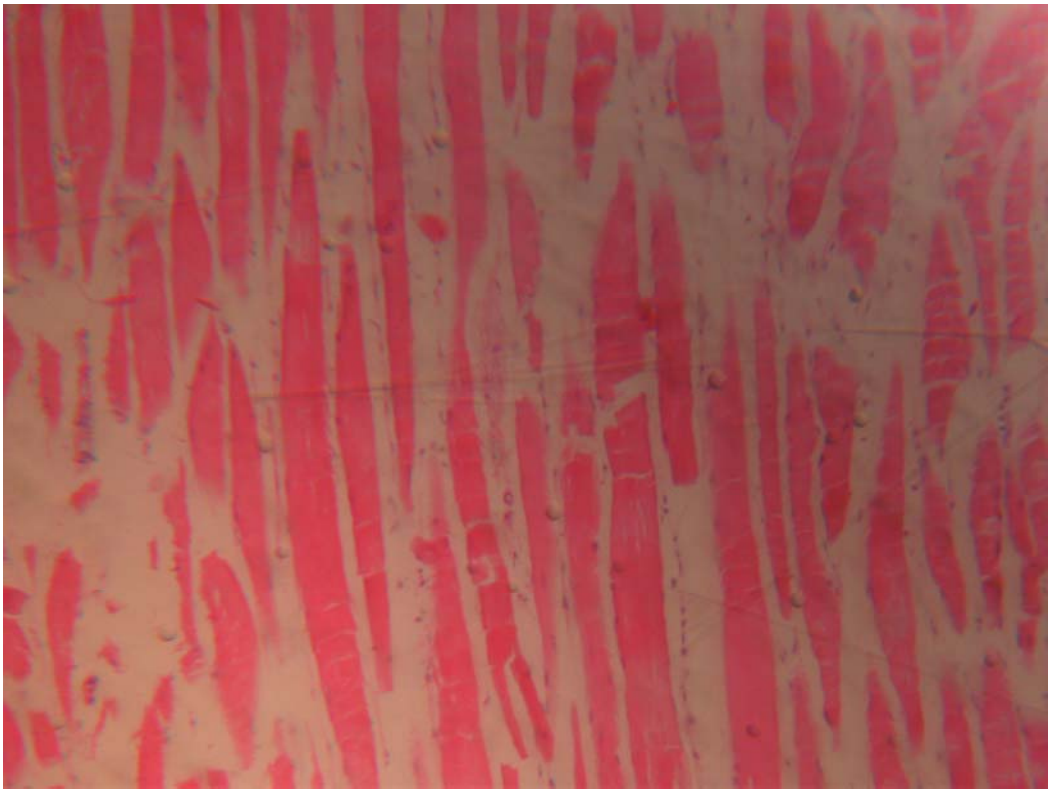


Fig. (4): Muscle from rats fed (1% cholesterol diet). Notice, fragmentation, hyalinization, necrosis and loss of muscle fibers nuclei. Haemotoxaline &Eusin(100 X)

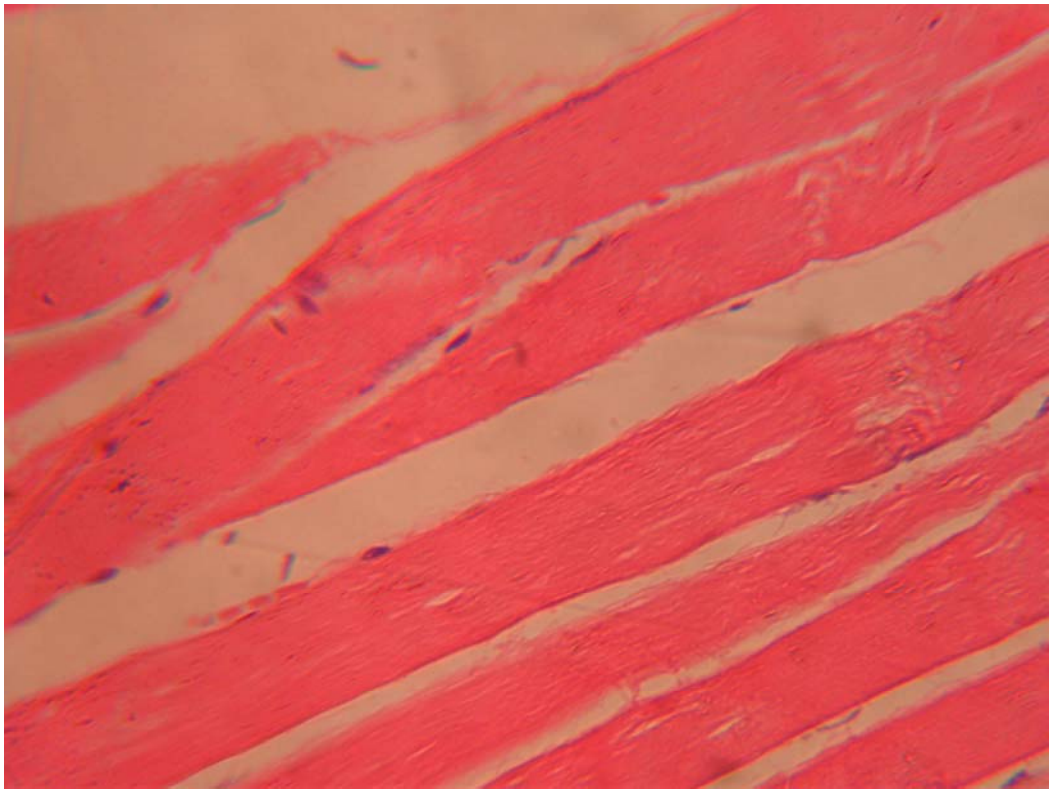


Fig (5): Muscle from rats received 40mg\kg Bwt Simvastatin. Notice less fragmented muscle fibers. Haemotoxaline &Eusin (400 X)

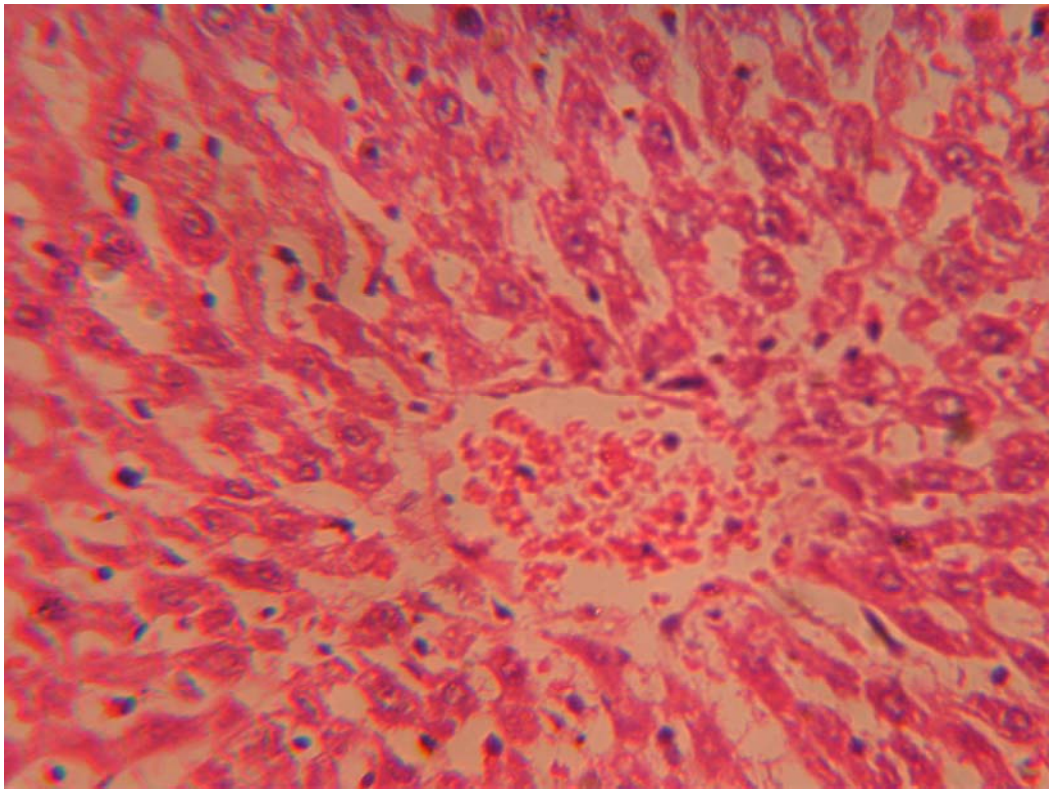


Fig. (6): Liver from rats received 80mg\kg Bwt Simvastatin. Notice, dilatation of sinusoids and congestion of the central vein. Haemotoxaline &Eusin(400 X)

CHAPTER FOUR
DISCUSSION

4.1 Induction of hypercholesterolemia and body weight

In this study, the addition of 1%cholesterol in diet for 2weeks significantly increased rat's body weight, and plasma cholesterol to level up to 193.33 mg/dL, this finding is on line with Son *et al.*, (2007) who found that, hypercholesterolemia can be induced on Wistar rats when they were fed a diet supplemented with cholesterol, the plasma cholesterol and body weight were significantly elevated in rats received a 1% cholesterol diet for 8 weeks. Also Getz *et al.* (2006) found that, Lipid-enriched diets are often used to induce or accelerate the rate of induction of persistent hypercholesterolemia in mice to levels > or approximately 300 mg/dL, as well as the protein source has been shown to influence lipoprotein level.

Birkner *et al.* (2009) showed that experimental hyper-cholesterolemia has been induced in the animals with the diet enriched with 0.5 and 2 g% of cholesterol/100 g of fodder/24 hours.

4.2Total cholesterol (T.C)

In this study, Simvastatin had a hypocholesterolemic effect in treated groups, it reduced cholesterol by 24.75%, 31.34%, 93.46% respectively, this agree with, Ragino (2008) experimentally in rabbit hypercholesterolemia. He found that at doses of 40, 66.5, and 100 mg/kg/day reduced total blood cholesterol by 36, 38, and 47% ($P<0.05$), respectively, after 20-

day. Also (Fraunberger *et al.*, 2009) found that simvastatin reduced circulating total-cholesterol and LDL- cholesterol levels by 68 % and 76 %, respectively.

4.2.1 Low density lipoproteins

The current study, showed that simvastatin had a significant effect on LDL-c .This result is on line with (Ballantyne *et al.*, 2008) who found that treatment with simvastatin reduced total cholesterol, total triglycerides and low-density lipoprotein cholesterol He also statin therapy alters the relationship between apolipoprotein B and low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol targets in high-risk patients.

Youssef, *et al.* (2004) repored that there was a significant reduction in T.C L and LDL cholesterol.

4.3 Serum constituents

4.3.1 Creatine kinase activity (CK)

In the present study, the activity of serum creatine kinase showed a significant increase in treated groups with simvastatin compared to control; this result agree with Chan, *et al.* (2005) who found that simvastatin was generally associated with high elevation and mild to moderate elevation of (creatine kinase) CK levels.

Myopathy is the most serious, dose dependent adverse reaction of statins and may lead to rhabdomyolysis and death. Clinical symptoms are muscle pain, weakness and muscle cramps, main laboratory finding is an increase in serum creatine kinase (CK). If myopathy symptoms are present or CK levels are greater than 5 times the upper limits of normal, statin therapy should be discontinued. In

case of an asymptomatic CK increase below 5 times the upper limits of normal, therapy can be continued with CK monitoring (Rasche-Schürmann *et al.*, 2008*)*

4.3.2 Liver Transaminases (ALT and AST)

The present study found that there was a significant changes in liver transaminiases on high dose simvastatin therapy, this result agree with, (Cohen *et al.* 2006) that demonstrated asymptomatic increases of liver enzymes the normal,have been reported in patients treated with simvastatin.

Dale *et al. (* 2007) found that more aggressive statin therapy increases the incidence of transaminase elevations.

On the other hand, (Dold *et al.*, 2009) who found that administration of 0.2 mg. kg $^{(-1)}$ simvastatin decreased liver enzymes ALT and AST by 87% and 83%, respectively, in mice.

CONCLUSION

In this study, it is confirmed that Simvastatin posseses a potent effect on Serum cholesterol and LDL-cholesterol and had undesirable effects on liver enzymes when it was given in high doses for along period of time “dose dependent”

Further research may be done to confirm this finding using different doses.

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